ANTENATAL RISK ASSESSMENT SCREENING FOR PREGNANCY ABNORMALITIES

This invention relates to a method for prenatal or antenatal screening for pregnancy abnormalities such as fetal (particularly chromosomal) abnormalities and to an apparatus for performing the method.

The risk of Downs Syndrome and some other chromosomal abnormalities in a fetus is known to increase with the age of the mother and it is this knowledge which forms the basis for selection of pregnant women for further investigation. Further investigation in the case of Downs Syndrome involves sampling of the amniotic fluid by amniocentesis, a procedure which itself carries a risk for the mother of the fetus, induction of a miscarriage being a recognised hazard of this procedure.

During pregnancy, maternal markers for Downs syndrome are widely used for screening, the most common being alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG) (either the intact molecule or free beta-subunit of hCG) and unconjugated oestriol (UE3). Disclosures relating to the use of such markers which may be used in combination with maternal age, include U.S. Pat. No. 4,874,693; WO 89/00696 and WO 90/08325.

Maternal screening is based on selecting a subgroup of women who are at the highest risk of giving birth to a child with an abnormality. In these women, the risk of invasive diagnostic procedures are considered to be outweighed by the risk of the abnormality. The risk is calculated by multiplying the a priori age related risk by the likelihood ratio The likelihood ratio is calculated from the relative heights of the multivariate Gaussian distribution functions of the marker analytes in Downs affected and unaffected normal pregnancies, corresponding to the value of the individual marker concentrations by dividing the height of the multivariate Gaussian distribution function for unaffected pregnancies by the height of the multivariate Gaussian distribution function for affected pregnancies.

As the concentrations of the analytes currently in use can vary normally with gestational age the analyte concentrations must be weighted accordingly. In turn with these analytes there is a relatively high dependence on accuracy of the estimation of age of gestation for the effective discrimination of Downs affected pregnancies. Weighting is performed by dividing the concentration of the analyte by the median concentration expected for that particular gestational age in women with unaffected pregnancies. This is termed the multiple of the median (MoM).

A combination of multiple analytes provides more information than any single analyte alone. The likelihood ratios determined from a multivariate combination is the most effective method of deriving information relating to the risk of a woman carrying a Downs affected child.

Inhibin is a dimeric molecule having alpha and beta-subunits covalently linked together via cysteine bridges. The alpha subunit is unique to the inhibin molecule and the beta-subunit has some homology with certain growth factors. In addition to dimeric inhibin, 'free' alpha-subunit forms (termed 'pro-alpha-N alpha C', 'pro-alpha-C' and 'alpha-C') and a beta-beta dimer (termed activin) are known to exist. To date only dimeric inhibin has been shown to confer biological activity and a biological activity for the alpha-subunit has yet to be elucidated. Moreover abundant amounts of immunoreactive alpha-subunit have been identified in biological fluids (Schneyer, Mason, Burton, Ziegner and Crowley, J. Clin. Endocrinol. Metab., 70, 1208–12, 990 and Lambert-Messerlian, Isaacson, Crowley, Sluss and Schneyer, J. Clin. Endocrinology and Metabolism, 78, 433–9, 1994) which are also known to contain immunoreactive dimeric inhibin (Knight, Groome and Beard, J. Endocrinology, 129, R9–R12, 1991).

The role of inhibin is unclear although there is a growing body of evidence that it may act as a regulator of pituitary gonadotrophin secretion or in a local paracrine/autocrine function with specific tissues (review Burger, Reproductive Medicine Reviews, 1, 1–20, 1992). It has been reported that immunoreactive 'alpha-inhibin' is secreted during the menstrual cycle (McLachlan, Robertson, Healy, Burger and de Kretser, J. Clin. Endocrinology and Metabolism, 65, 954–61, 1987), in response to exogenous gonadotrophin stimulation during artificially controlled cycles in women (McLachlon, Robertson, Healy, Burger and de Krestser 1987 and Robertson, Fertil. Steril. 48, 1001–08, 1987) and by the fetal placenta during pregnancy (Tovanabutra, Illingworth, Ledger, Glasier and Baird, Clin. Endocrinology, 38, 101–7, 1993). The term 'immunoreactive alpha-inhibin' is used in this context because all the inhibin assays employed in these studies and the Downs studies described below were either alpha-subunit specific by definition or preferentially cross-react with the free forms of inhibin alpha-subunit (Lambert-Messerlian, Isaacson, Crowley, Sluss and Schneyer, 1994). It is therefore unlikely that immunoreactive alpha-inhibin levels reflect immunoreactive dimeric inhibin levels.

Other studies have investigated 'alpha-inhibin' as a potential marker in maternal serum for the presence of Downs syndrome in the unborn child (van Lith, Pratt, Beekhuis and Mantingh, Prenatal Diagnosis, 12, 801–6, 1992; Spencer, Wood and Anthonyyr Anal. Clin. Biochem., 30, 219–20, 1993, Cuckle, Holding and Jones, Prenantal Diagnosis, 14, 387–90, 1993). At the 5% false-positive detection rate, only 40% of the affected pregnancies were detected by combining alpha-inhibin concentrations with maternal age (van Lith, Pratt, Beekhuis and Mantingh, 1992) and alpha-inhibin concentrations were also highly correlated with free beta-hCG levels (Spencer, Wood and Anthony, 1993) and intact hCG (Cuckle, Holding and Jones, 1993). As a result it was concluded that these findings were likely to argue against the use of alpha-inhibin immunoreactivity as an additional biochemical marker in Downs syndrome screening programmes (Spencer, Wood and Anthony, 1993). Whilst Cuckle et al, 1994 refer to such use as "of limited value".

According to the present invention we provide a method for antenatal screening for pregnancy abnormalities such as fetal (particularly chromosomal) abnormalities in which a sample of maternal body fluid from a pregnant woman is measured for the level of at least one marker and/or a precursor or metabolite of said marker and the measured level of this marker together with the gestational age of the woman are compared with reference values at various gestational ages of the level for the marker in (a) pregnant women carrying fetuses having abnormality(s) subject to the screen and/or b) pregnant women carrying normal fetuses, the comparison being indicative of the risk of the pregnant woman carrying a fetus with an abnormality subject to the screen characterized in that the marker is dimeric inhibin.

Further according to the invention we provide an apparatus comprising means adapted for receiving measurement of a pregnant woman's maternal body fluid level of at least one marker and/or a precursor or metabolite of said marker and computer means for comparing the measurements of this level to sets of reference data to determine pregnancy abnormalities such as fetal (particularly chromosomal) abnormalities characterised in that the marker is dimeric inhibin.

In particular the invention relates to the use of an assay that is capable of discriminating between dimeric inhibin from other inhibin-related proteins such as inhibin alpha-subunit in maternal serum. Such an assay employs antibodies which bind to two unique binding sites expressed on the